Gastroduodenal reflux: the role of trypsin in lung deterioration after lung transplantation

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Background

•Bronchiolitis obliterans syndrome (BOS) is the main limitation against the long-term survival of lung allografts
•The development of BOS is associated with gastro-oesophageal reflux disease (GORD)[1] which is commonly found in lung transplant patients
•Pepsin and bile acids, non-acid components of refluxate have been studied as good predictors of the development of BOS[2].
•Trypsin (a duodenal protease) can be detected in refluxate
•Therefore trypsin in gastro-duodenal refluxate is another potential damaging agent

Aims

•The aims of this project were:
- •To develop a more sensitive method of detecting trypsin in refluxates
- •To analyse the viability of trypsin as a predictor of BOS

Methods

•A sandwich ELISA protocol was optimised for enhanced sensitivity by increasing capture and detection antibody concentrations. The initial protocol had capture, detection and secondary antibody dilutions of 1:2000, 1:2000 and 1:5000 respectively.
•100µg/ml trypsin was subjected to artificial gastric conditions for times ranging from 0-180 minutes (three hours). N-terminal activity assays were run for these trypsin samples.

References

1. King B.J., Iyer H., Leidi A.A., Carby M.R. (2009) 'Gastroesophageal reflux in bronchiolitis obliterans syndrome: a new perspective.' , *J Heart Lung Transplant*, 8, (9), pp. 870-5.
2. Blondeau, K., Mertens, V., Vanaudenaerde, B.A., Verleden, G.M., Van Raemdonck, D.E., Sifrim, D., Dupont, L.J. (2008) 'Gastro-oesophageal reflux and gastric aspiration in lung transplant patients with or without chronic rejection', *Eur Respir J*., 31, (4), pp.707-13.
3. Gotley, D.C., Morgan, A.P., Ball, D., Owen, R.W., Cooper, M.J. (1991) 'Composition of gastro-oesophageal refluxate.', *Gut,* 32, (10), pp. 1093-9.
4. Tack, J. (2005) 'Review article: role of pepsin and bile in gastro-oesophageal reflux disease.', *Aliment Pharmacol Ther.*, 22, (Suppl 1), pp. 48-54.

Results

1. It is possible to detect trypsin at levels as low as 0.05µg/ml by increasing capture and detection antibody concentrations.

A)

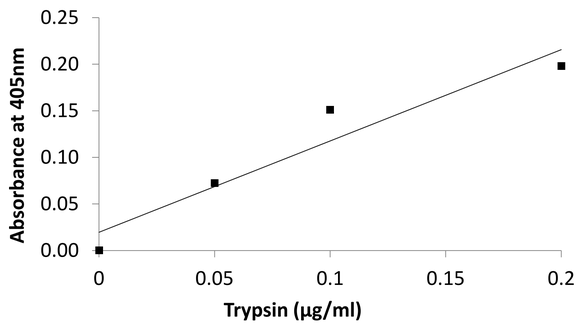
Capture antibody dilutions	1:500	1:1000	1:2000
Percentage absorption*	153	130	100

B)

Detection antibody dilutions	1:500	1:1000	1:2000
Percentage absorption*	484	182	130

* Percentage increase in absorbance for standard curve trypsin values

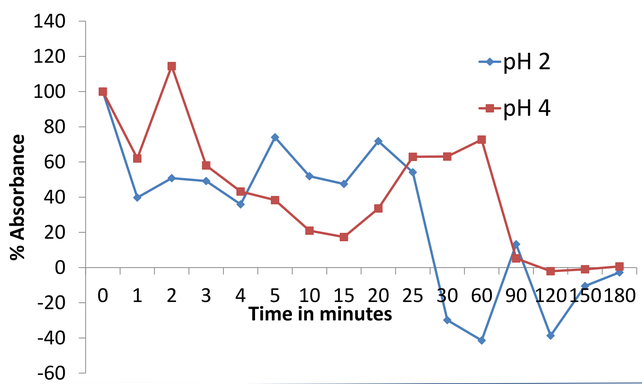
C)



1: ELISA optimisation. Increasing capture antibody can give increased absorbance readings(A). Combining this with secondary antibody concentration increase can give even higher absorbance readings (B). Using capture and detection antibody dilutions both of 1:1000, trypsin can be detected at levels as low as 0.05µg/ml or 50ng/ml (C).

2: Trypsin activity assay. Trypsin will eventually lose its activity in the gastric environment. When this occurs depends on the pH of the gastric environment with higher pHs supporting a retention of trypsin activity for longer periods of time.

2. Trypsin maintains its activity after passing through the gastric environment in a pH-dependent manner.



Discussion

•It has been found that a combination of pepsin and bile acids could cause lethal damage to lung allografts at acidic pH ranges[4].
•Trypsin has the potential to cause damage at weakly acidic and neutral pHs, at which it is active, in a mechanism similar to that of pepsin.
•Not all cases of GORD occur at acidic pHs.
•This project reveals that trypsin retains its activity at weakly acidic pHs and so can cause damage to lung allografts. It also proposes a sensitive method of detection.
•Future work will involve testing the newly developed protocol on BAL and sputum samples to see if it can detect trypsin post-gastric transit.

Conclusion

•Low concentrations of trypsin can be detected using sandwich ELISA
•Trypsin retains its activity after passing through the stomach for varying periods of time which is pH-dependent
•Hence, trypsin could be a viable as a marker of gastro-duodenal reflux entering the lungs